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CARBONYLS IN OXIDIZING FAT. I. SEPARATION OF STEAM VOLATILE MONOCARBONYLS INTO CLASSES[a]

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Carbonyl compounds influence the flavor and quality of many foods and natural products. They are believed to be largely responsible for the rancid flavors developed in oxidizing fat and fatty tissue. However, relatively little is known concerning the influence of composition, treatments, and conditions on the kinds and amounts of carbonyls formed in fat, and their relationship to odor and flavor *(11, 18, 23, 30, 38)*. The objectives of this work were to determine some of the changes occurring in fractions of the steam volatile carbonyls of freezer-stored pork fat.

There is considerable need for a more specific objective method of determining rancidity. The shortcomings of the organoleptic methods *(1, 2, 21, 34, 35)* have been fully described elsewhere *(4, 24, 36, 47)*. Peroxide values can be used to follow the amount of oxidation, but they do not constitute a specific determination of rancidity *(31, 32, 33, 36, 38)*. Rancidity has been found to appear at different peroxide levels. Carbonyls are produced as a result of the decomposition of the peroxides, and apparently their nature depends on the kind of hydroperoxides, conditions, and other variables *(38)*.

In recent years the sensitivity of methods involving 2, 4-dinitro-phenylhydrazone[b] derivatives of carbonyls has been applied. Pool and Klose *(40)* have devised a method for determining total monocarbonyls, Neumer and Dugan *(37)* have reported a procedure for total volatile carbonyls, and Chang and Kummerow *(10)* a method for total and volatile carbonyls. In general, applications of the above methods have indicated a close relationship between peroxides and carbonyl values. Total carbonyl values, therefore, also appear not to be specific for rancidity.

Consideration of these facts, together with the well-known sensitivity of taste, leads to the assumption that rancid flavors may be due to some particular type of carbonyls *(8, 9, 14, 15, 27)*, the proportions and rate of formation of which are influenced by the composition of the glycerides, catalysts, enzymes, and anti-oxidants present, and processing and storage conditions. Henick, Benca, and Mitchell *(23)* have developed a method for determination of total saturated and unsaturated carbonyls as hexanal and crotonaldehyde. Applications *(23, 38)* of this method showed the rate of increase of saturated carbonyls exceeded that of the 2-enal class. The method does not take into account other classes of carbonyls that may be present.

In studies of autoxidative mechanisms *(6, 8, 9, 22, 25, 27, 28, 44, 45, 46)* one or more compounds of the following classes have been identified: saturated aliphatic aldehydes, saturated ketones, 2-en-1-als, 2, 4-dienals, and dicarbonyls.

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[b] Hereinafter referred to as DNPH.

A number of investigators have recently reported identification of carbonyls in food products *(12, 14, 15, 39, 48)*. A quantity of individual compounds of the expected types of carbonyls were found by Forss, Pont, and Stark *(14, 15)* in off-flavored skim milk, and by Pippen, Nonaka, Jones and Stitt *(39)* in a simmering mixture of chicken muscle tissue and water. For the most part, carbonyls were isolated from large amounts of material, and DNPH derivatives obtained in large enough quantity to permit separation by repeated column adsorption chromatography, and identification by conventional procedures. Such methods are laborious and time-consuming and are not suited to the following of qualitative and quantitative changes during oxidation.

This paper describes the application of procedures for the separation and determination of total volatile carbonyls, monocarbonyls, and dicarbonyls of pork fat. The volatile monocarbonyls were separated into classes by a rapid new paper chromatographic procedure *(19)* for separation of mixtures of 2-alkanone, alkanal, alk-2-enal, and alk-2,4-dienal DNPH derivatives. These procedures were referred to in a recent note *(18)* which described the identification of volatile saturated aliphatic aldehydes and methyl ketones in a rancid pork fat.

The new method for separation of homologous series of 2-alkanones, alkanals, alk-2-enals, and alk-2,4-dienals into classes described by Gaddis and Ellis *(19)* fails only with respect to acetone, methanol, and ethanal. Acetone separates in the alkanal class, and methanol and ethanal come out in the alk-2-enal class *(19)*. As a result, when the classes are separated into individual compounds *(13)*, mixtures of ethanal-acrolein, acetone-propanal, and acetone-butanal are obtained *(19)*. However, the presence of acetone, methanol, and ethanal in the mixture of monocarbonyl DNPH's can be detected by the partial extraction of these compounds from carbon tetrachloride with a 2 N HCl solution of 2,4-dinitrophenylhydrazine *(18)*. Spectrophotometric studies in neutral solution and in alcoholic alkali *(13, 26)* are useful in the detection of such mixtures, and where necessary corrections can be applied. Over-all, the methods of Ellis, Gaddis, and Currie *(13)* and Gaddis and Ellis *(19)* result in a powerful tool for rapid identification and estimation of classes and individual compounds. In the freezer-stored pork fat *(18)* examined so far, acrolein has not been detected, and methanol, ethanal, acetone, and methyl ethyl ketone are usually present only in trace amounts. These compounds are in part removed from the other monocarbonyls by the extraction procedure. Hexanal and propanal were identified as the major saturated compounds.

EXPERIMENTAL

Solvents and reagents. A. C. S. grades of carbon tetrachloride, benzene, methanol, and commercial absolute ethanol, Skellysolve C[e] (b 91° —95°) were purified as described by Ellis, Gaddis, and Currie *(13)*. Technical grade petroleum ether was distilled and the fraction boiling 37° —40° C.

[e] Mention of specific commercial materials or equipment throughout this paper does not constitute recommendation for their use above similar materials and equipment of equal value.

was collected for use. Alcoholic potassium hydroxide (0.25 N) was prepared as described by Ellis, Gaddis, and Currie *(13)*. Tetrahydrofuran was redistilled. A. C. S. grade anhydrous ether; propylene glycol U. S. P.; and vaseline (white petroleum jelly) Blue seal, Cheesbrough Manufacturing Company, were used without further treatment. The 2,4-dinitrophenylhydrazine reagent was prepared by grinding the 2,4-dinitrophenylhydrazine [d] in a mortar with 2N HCl and filtering. This gave a solution containing approximately 5 mg. DNP per ml. The solution was purified by shaking with carbon tetrachloride in a ratio of 1 to 5, separating and filtering *(3)*. This reagent was prepared fresh daily.

Materials and equipment. Fat samples were steam distilled in detachable 32 x 300 mm. culture tubes connected to a micro Kjeldahl-type steam distillation apparatus. Alcoa alumina (grade F-20) was modified according to Pool and Klose *(40)* by mixing with 20% of hydrated alumina. Whatman No. 3 filter paper sheets were cut in tapering strips as described by Ellis, Gaddis, and Currie *(13)*. Similarly, 38 × 300 mm. culture tube chromatographic chambers, with pressure regulation by mercury traps, were employed *(13)* in ascending solvent development. Spectrophotometric measurements were made with a Beckman Model DU, using a tungsten lamp as light source.

Procedure. Ground fat tissue was rendered at as low a temperature as possible on the steam bath. As fast as formed, the melted fat was filtered through cheesecloth and 10.0 g. portions weighed into 32 × 300 mm. culture tubes. Two determinations were usually made on each sample of tissue fat — on the rendered fat, and on the rendered fat after heating at 165° C. for 15 minutes to approximate the effect of cooking. Carbonyls were volatilized by steam for 22 minutes at a distilling rate of 6 ml. per minute. The distillate was collected in an ice-cooled flask containing 50 ml. of 2N HCl solution of DNP, composed of 10 to 50 ml. of saturated DNP reagent. During this operation, the condenser tip was immersed in the reagent, and, when the steam distillation was completed, the tip was washed inside and out with carbon tetrachloride. In some experiments, carbonyls which volatilized during the heating (cooking) process at 165° C. were collected and reacted with the DNP reagent in a similar manner. The steam distillate reaction mixtures were allowed to stand overnight at room temperature. The cloudy solution, or suspension, of DNPH'S was extracted with carbon tetrachloride and then benzene. In this operation both extracts were suitably washed with 2N HCl and then water. The extracts were dried over powdered anhydrous sodium sulfate and filtered, and, when total carbonyls were measured, the carbon tetrachloride extracts were made to 100 ml. Solvent was removed from the benzene extracts on the steam bath with a jet of nitrogen gas, and residues made to a suitable volume with carbon tetrachloride. Determination of total carbonyls were made with the spectrophotometer by measurement of the absorbance at the maxima.

The total carbonyls were separated into monocarbonyls and dicarbonyls, and freed of excess DNP by chromatography on 0.35" × 2.5" columns of 20% hydrated alumina. Solvent was removed from the two fractions on a steam bath with a jet of nitrogen gas. The two residues were dissolved in minimum quantities of carbon tetrachloride and applied to separate columns of alumina, prepared by adding about ¼ in. of powdered anhydrous sodium sulfate and passing through a preliminary portion of benzene. The monocarbonyls were separated and eluted with benzene. After development with ether, the dicarbonyls were eluted with ethanal. Solvents were removed from the monocarbonyl and dicarbonyl fractions and the residues dissolved in carbon tetrachloride and made to a convenient volume. The absorbance at the wave length of maximum absorption was determined for each fraction.

The volatile monocarbonyls were resolved into classes by a rapid paper chromatographic procedure *(19)*. Mechanically, this technique was the same as that described by Ellis,

[d] Hereinafter referred to as DNP.

Gaddis, and Currie *(13)*. In order to compare fractions, on the basis of proportions of classes, the same quantities of total coloring components were spotted. Amounts spotted were calculated by the following formula:

$$\text{X ml. of carbonyl DNPH solution} = \frac{1.5\ \text{(monocarbonyls)}}{\text{absorbance of carbonyl DNPH solution}}$$

The aliquot thus determined was evaporated on the steam bath with a jet of nitrogen gas to a suitable volume and spotted on the paper. Each separation was run in groups of 3 strips or more in order to obtain enough material for characterization of the separated spots by spectral analysis. Extracted material was dissolved in 3.00 ml. carbon tetrachloride and the absorbance determined. Occasional determinations were also made in alcoholic alkali. This was usually done by removal of the solvent from the sample read in neutral solution, adding 0.30 ml. of benzene and 2.70 ml. of alcoholic potassium hydroxide, mixing and reading immediately. Preliminary paper chromatographic separation of the dicarbonyl fraction was also made, using a similar technique. Paper chromatograms were run at room temperature, using a mixture of 5.00 ml. of Skellysolve C and 1.00 ml. of tetrahydrofuran as ascending solvent. This solvent combination was suggested by Rice, Kellar, and Kirchner *(41)*.

Paper chromatographic separation of the classes of monocarbonyls into their individual compounds was made by the method of Ellis, Gaddis, and Currie *(13)* and Gaddis and Ellis *(19)*.

Peroxides were determined on the rendered fat by the method of Rockwood, Ramsbottom, and Mehlensbacker *(42)*.

RESULTS AND DISCUSSION

Carbonyl values. Total carbonyl, and mono- and dicarbonyl values, were determined for a considerable quantity of freezer-stored cured and uncured pork tissue samples from different animals. Statistical analysis of the data indicated a close association with peroxide values. This agrees with the findings of the few investigators *(29, 37)* who have examined the relationship. However, study of the data indicated that the high association may be somewhat superficial in nature. Total and monocarbonyl values often varied considerably at similar peroxide levels. The work of Nikkila and Linko *(38)* on vegetable oils indicated that peroxide-carbonyl relationships may differ in oils and fats of different origin and composition. The existence of differences in peroxide-carbonyl relationships would tend partly to explain the detection of rancidity at different peroxide levels *(36)*.

Data plotted in Figure 1 for a differently oxidized group of unrelated lard samples indicated variations in peroxide-carbonyl relationships. The greatest difference or variability was in the heated samples, and appeared to involve the monocarbonyl fraction. The dicarbonyl fraction showed a linear relationship with peroxides. The variation possibly was due to differences in composition and stability of the lard samples.

Data shown in Figure 2 indicate peroxide-mono- and dicarbonyl relationships found for uncured and cured tissue samples from the same hog. (In this experiment, one bacon side was dry-cured with 4% salt and both sides were cut into six equal segments. The segments were stored at 15° F. and tested after 18 and 36 weeks of storage. Adjacent segments were distributed between the two storage periods. The first 3 points in each curve represent segments from 18 weeks and the last three from 36 weeks storage.) In a given group of samples, peroxide-monocarbonyl relationships tended to be linear in the early stages of oxidation. However, a difference in that relationship was indicated between uncured and cured samples. This was apparently

due to the pro-oxidant effect of the salt. There was little difference in the dicarbonyls of the two sets of samples, and relationships with peroxides were linear except in advanced stages of oxidation. Further work is required on the influence of composition, anti-oxidants, pro-oxidants, and other factors on peroxide-carbonyl associations. Such investigations seem necessary for a complete understanding of the chemistry of rancidity.

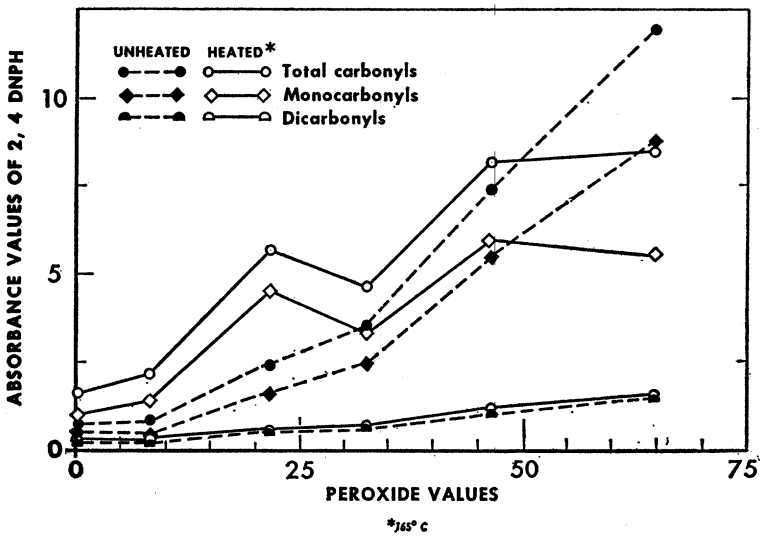

Figure 1. Oxidized lard.

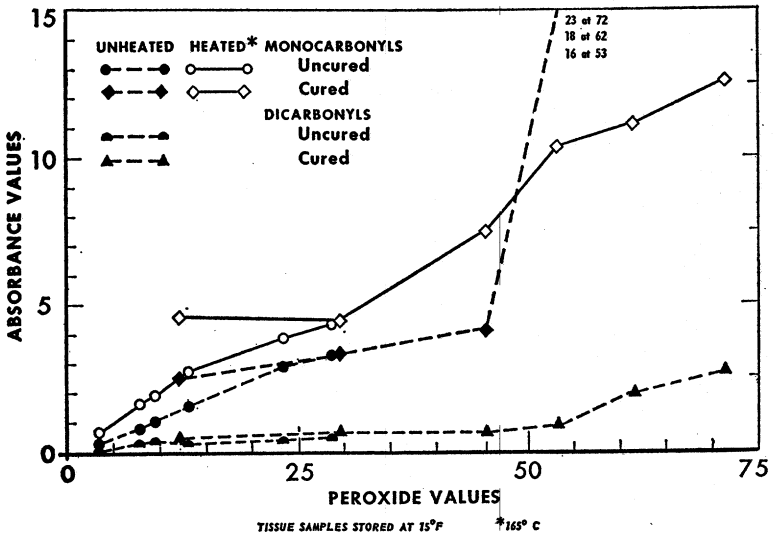

Figure 2. Paired bacon sides.

As indicated in an earlier report *(17)* total carbonyls of heated fat (Figure 1) increased at a more rapid rate than those of the unheated fat during the early stages of oxidation. This was also shown to be true in Figures 1 and 2 for monocarbonyls. However, in advanced oxidation, formation of carbonyls of unheated fat overtook and exceeded those of the heated fat. This curious behavior is probably partly due to volatilization and to decomposition, but it also appears to be chiefly a function of the degree of oxidation.

The monocarbonyl DNPH's gave the characteristic red color with alcoholic alkali. As reported earlier *(18)* the very small amount of monocarbonyls in the benzene extract so far have been found to consist entirely of low molecular weight saturated monocarbonyls. The dicarbonyl fractions gave blue colors in alcoholic alkali, which is characteristic of α dicarbonyls.

In general, the wavelength of maximum absorption of the unheated monocarbonyl fractions decreased with oxidation from 355 mμ to 343-6 mμ, which indicated an increase in the proportion of saturated constituents. However, the wavelength of maximum absorption of the heated monocarbonyl fractions increased from 355 mμ to 365 mμ with oxidation. This shift indicated increases in proportions of unsaturated compounds.

Separation and identification of monocarbonyl classes. Paper chromatography of the monocarbonyls gave cleancut separations into 2 to 3 spots. Maxima were generally sharp and corresponded to those characteristic for saturated aldehyde, 2-en-1-al, and 2,4-dien-1-al classes. Braude and Jones *(5)* and others *(14, 15, 26)* have determined the characteristic range of maxima in ethanal and chloroform of the classes of 2,4-DNPH's. These values, along with determinations by the authors in carbon tetrachloride, ethanal, and alcoholic alkali, are shown in Table 1.

A spot was obtained from unheated fat and low oxidized heated fat that has not been completely identified. This fraction had maxima of 349-58 mμ, which is intermediate between the saturated aldehydes and 2-en-1-als. This fraction was discussed by Gaddis and Ellis *(18)*, and the possibility considered that it might contain unconjugated unsaturated aldehydes. The R_F's of this fraction were nearly identical with those of the 2-en-1-al class. It is now considered to be a mixture of methanol, ethanal, 2-en-1-als, and unknown compounds. The fraction will be further discussed below.

Table 2 shows a comparison of R_F values obtained in the separation of mixtures of authentic DNPH's of as much as four classes of monocarbonyls, and the monocarbonyls from a sample of heated fat. This illustrates the effectiveness of the separation, with compounds separating into their proper classes with the exception of acetone, methanol, and ethanal *(19)*.

Changes in the proportions of monocarbonyl classes. Proportions of the different classes were determined by measurement of the absorbance values at the maxima, and calculation as per cent of the total amount recovered. Figures 3 and 4 show changes with oxidation in the major classes, in saturated aldehydes, and in 2,4-dienals of four different groups of samples. The proportion of saturated aldehydes (Figure 3) increased rapidly *(23, 28)*. However, in the heated fat, proportions of the saturated class generally decreased, but in late stages of oxidation showed a tendency to increase again slightly. Early in the oxidation, the 2,4-dienal class frequently was not detected, and heated monocarbonyls were similar in composition to those of the unheated.

TABLE 1

Absorption maxima of classes of carbonyl 2,4-dinitrophenylhydrazones

	Authors			Braude and Jones		Jones et al. (26)		Forss et al. (14, 15)
	CCl_4 mμ	EtOH mμ	Alc. KOH mμ	$CHCl_3$ mμ	EtOH mμ	$CHCl_3$ mμ	Alc. KOH mμ	EtOH mμ
Aliphatic saturated aldehydes	330-49	346-58	420-34	348-61	348-63	344-58	426-30	357-8
Saturated methyl ketones	349-52	358-61	434	365-8	362-5	364-7	430-34	——
2-en-1-als [1]	358-65	370-5	456-60	367-73	366-73	373	452-59	370-5
2,4-dien-1-als [1]	380	389-90	480-4	388	379-85	——		389
Mono α dicarbonyl				352	351			
Bis α dicarbonyl				400				
Bis, unconjugated dicarbonyl				366				
Unknown dicarbonyls	380-5		545.50					
Diacetyl		395*						

* Diacetyl in $CHCl_3$

[1] Furnished by E. L. Pippen, WURDD.

TABLE 2

Comparison of separations into classes by paper chromatography at 4° C.

2,4-DNPH's	R_F values				Max.
	1	2	3	Av.	mμ
Mixture					
Saturated aldehydes, C_3-C_{14}	0.47	0.48	0.47	0.47	See Table 1
2-en-l-als, C_3-C_{12}	0.37	0.36	0.35	0.36	
2,4-dien-l-als, C_5-C_{12}	0.21	0.23	0.23	0.22	
Monocarbonyls					
From heated	0.48	0.48	0.48	0.48	346
Autoxidized stored (0° F.)	0.35	0.37	0.36	0.36	361
Tissue fat	0.21	0.24	0.23	0.23	375-80
Mixture					
Methyl ketones, C_5-C_9	0.58	0.56	0.57	0.57	See Table 1
2-en-l-als, C_3-C_{12}	0.33	0.33	0.33	0.33	
2,4-dien-l-als, C_5-C_{12}	0.21	0.20	0.20	0.20	
Mixture					
Methyl ketones, C_5-C_9	0.60	0.58	0.59	0.59	See Table 1
Saturated aldehydes, C_1-C_{14}	0.42	0.42	0.42	0.42	
2-en-l-als, C_3-C_{12}	0.32	0.30	0.31	0.31	
2,4-dien-l-als, C_5-C_{12}	0.17	0.17	0.17	0.17	

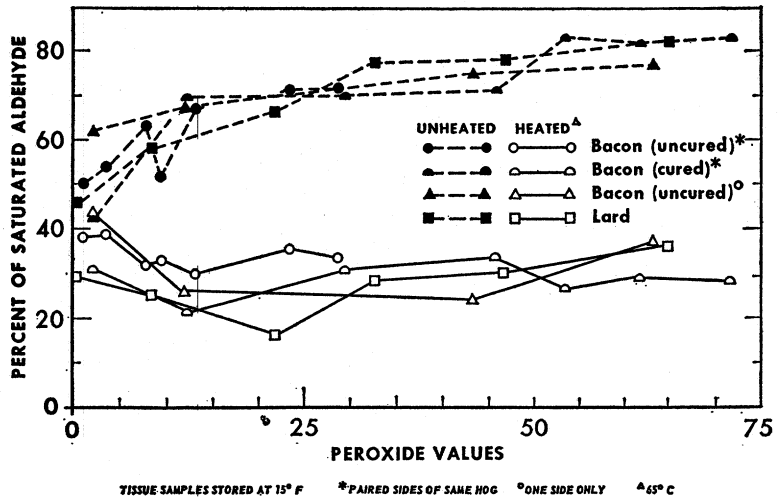

Figure 3. Saturated aldehydes. Changes with oxidation.

With oxidation the proportions of the 2,4-dienal class increased rapidly in the heated monocarbonyls. However, this class tended to decrease somewhat with advanced oxidation. There was considerable difference in the behavior of the different groups of samples. Interpretation of this requires further

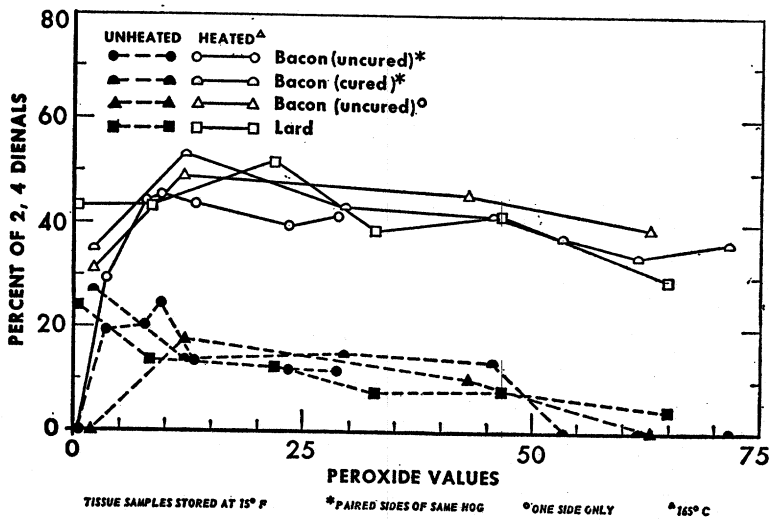

Figure 4. 2,4 dienal. Changes with oxidation.

study. Perhaps the greatest significance of these data is the rapid change in proportions that takes place during the initial stages of oxidation at the time rancidity first develops. Proportions of saturated aldehydes changed from 50% to 85% in the unheated, and 2,4-dienals from 0% to 55% in the heated fat.

Oxidation and relative quantities of monocarbonyl classes. The absolute absorbance values of the classes were determined from the total monocarbonyl absorbance values and the percentage proportions of the classes. Data shown in Figures 5, 6, and 7 are an extension of the experiments covered

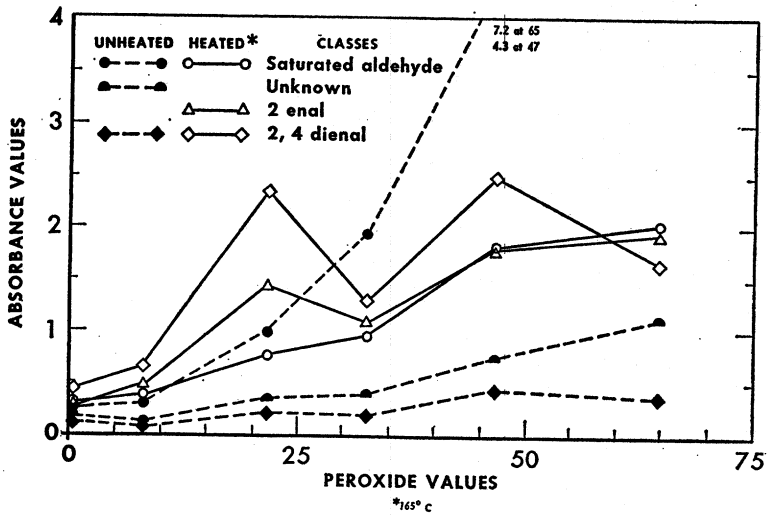

Figure 5. Lard. Oxidation and relative amounts of monocarbonyls.

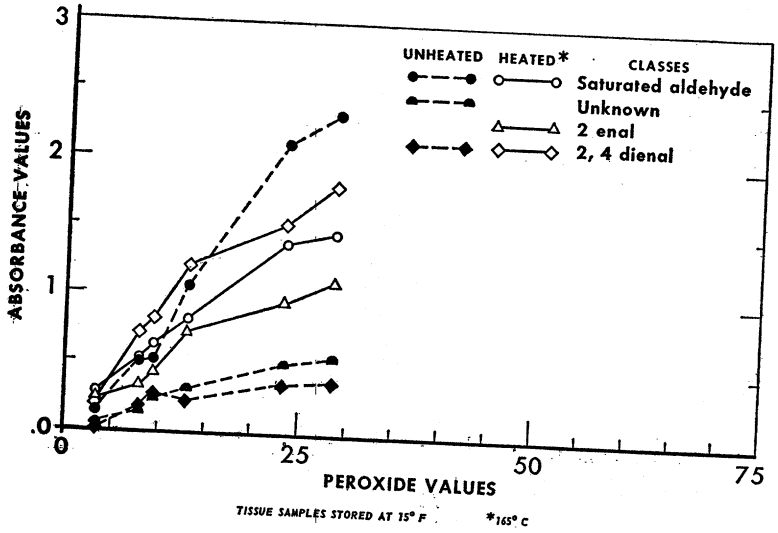

Figure 6. Uncured pork fatty tissue. Oxidation and relative amounts of monocarbonyls.

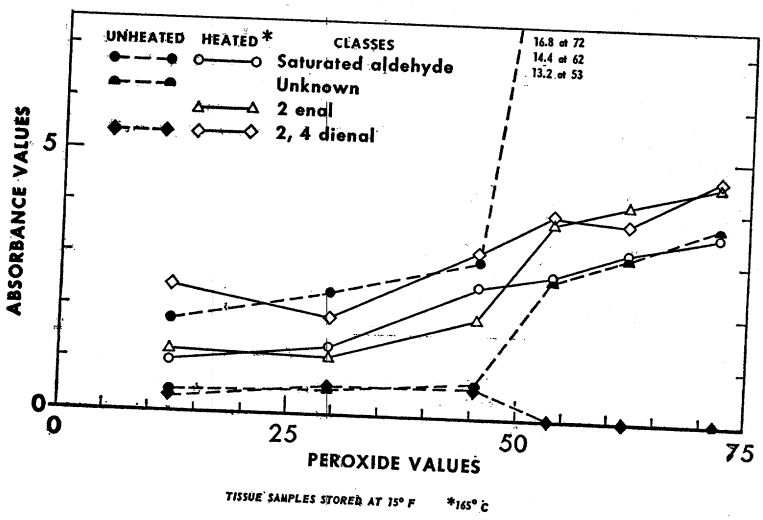

Figure 7. Cured pork fatty tissue. Oxidation and relative amounts of monocarbonyls.

by Figures 1 and 2. The absolute absorbance values are plotted against the peroxide values.

In Figure 5, the erratic behavior of the heated total and monocarbonyl discussed in connection with Figure 1 is indicated to be due primarily to the generation of 2,4-dienals in a manner unpredictable on the basis of the peroxides. The heated 2-enals also showed some variation. The rest of the class curves were fairly smooth.

In Figure 6, similar data for uncured bacon are given. The samples were from the same bacon side, and all monocarbonyl classes increased smoothly

with oxidation. These data illustrate particularly well the rapid increase and change in relative amounts of the monocarbonyl classes during the early stages of oxidation. Data shown in Figure 7 for cured bacon from the same animal (see Figure 7) are considerably different, although the fat was presumably similar in composition .Comparing Figures 6 and 7 over the same range of oxidation (peroxide 12-30), the rate of change and relative amounts of the classes are obviously different. It would appear that the pro-oxidant, salt, may influence the formation of oxidation products in some manner.

In over-all consideration of Figures 5, 6, and 7, there was a progressively increasing quantitative difference between the unheated and heated saturated classes. Although a large proportion of this class is composed of low molecular weight volatile compounds, volatilization during heating did not account for the decrease. Since the unheated saturated class increased tremendously in the late stages of oxidation, the disappearance on heating becomes very great, and probably accounts for the previously discussed reversal in quantitative relationship of the unheated and heated total monocarbonyls. Determination of the influence of fat composition and other variables on the relative amounts of the monocarbonyl classes would appear to be necessary for an understanding of the chemical nature of rancidity.

Quantitative aspects of carbonyl determination. A thorough study has been made of the reproducibility of the above determinations. This was found to usually not exceed ±3%. In the separation into classes, as shown by Gaddis and Ellis *(19)*, results for the 2,4-dienals will tend to be somewhat low and the other classes a little high. This is due to the particular sensitivity of the 2,4-dienals to light and air.

Separation of classes into individual compounds. Data shown in Figures 8 and 9 illustrate the separation by ascending paper chromatography *(13, 19)*

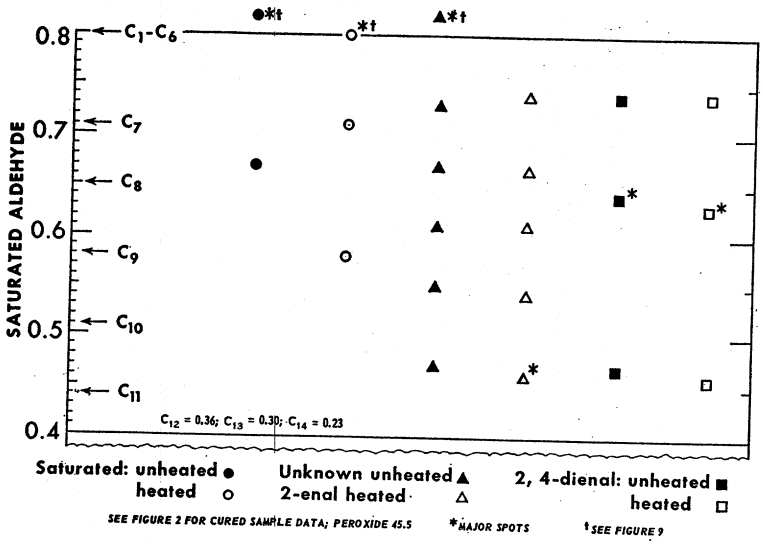

Figure 8. Vaseline-aqueous methanol system. Separation of classes into compounds, R_f values.

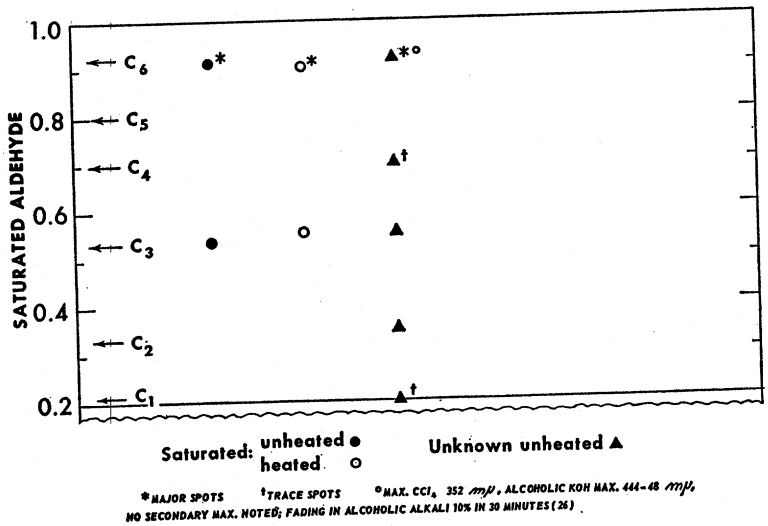

Figure 9. Propylene glycol, Skellysolve C-Methanol. R_f values of unseparated material from vaseline-methanol system.

of the different classes of a sample of unheated and heated cured pork fat into compounds. On the left-hand side, the scale indicates R_F values and the adjacent column a homologous series of authentic saturated aldehyde DNPH's. Unknown DNPH mixtures were applied first to the vaseline-aqueous methanol system (Figure 8), and after chromatography the unseparated material was transferred to the propylene glycol, Skellysolve C-methanol system (Figure 9). As indicated in Figure 8, higher molecular weight carbonyls were apparently similar in unheated and heated fats, with the exception of the saturated aldehydes. In that class, the unheated contained one compound and the heated two compounds. In the separation of lower molecular weight compounds shown in Figure 9, the heated contained none in the 2-enal class and was similar to the unheated in the saturated aldehyde class. The saturated aldehydes have been previously identified *(18)* as hexanal, propanal, ethanal, and methanal. Traces of actone and methyl ethyl ketone were also found. The two compounds with lowest R_F values in the unknown class are unquestionably methanal and ethanal *(19)*. However, the ethanal might contain acrolein and the propanal traces of acetone. These results indicate the presence of 6 saturated aldehydes, two methyl ketones, three unknown unsaturated compounds, five 2-enals, and three 2,4-dienals; a total of at least 19 monocarbonyls in oxidized pork fat.

Dicarbonyls. The solubility of the dicarbonyl fractions (Figures 1 and 2) in common organic solvents indicated they are probably mostly composed of compounds of relatively long carbon chains. The fractions had maxima in carbon tetrachloride from 350-90 mμ. Maxima of unheated dicarbonyls were generally in the range of 380-90 mμ and showed a secondary peak at 430-40 mμ. Dicarbonyls from heated fat often showed a down shift to about 350-65mμ. According to Braude and Jones (5) (see Table 1) bis hydrazones

have normal absorption except those of α diketones which have maxima as high as poly-unsaturated monocarbonyls. Apparently the downward shift in maxima indicates the presence of small amounts of unconjugated dicarbonyls. Paper chromatography *(41)* gave three main fractions with maxima at 370, 385, and 390 mμ in carbon tetrachloride. These gave purplish-blue to blue colors in alcoholic alkali with maxima at 525, 545, and 550 mμ. Several minor fractions with maxima at 346 to 358 mμ preceded the α-dicarbonyls on the paper.

SUMMARY

Methods are described for the estimation in fat of total steam volatile carbonyls, and mono- and dicarbonyl fractions thereof, as DNPH derivatives. The application of a new paper chromatographic method to the separation and determination of classes of monocarbonyl DNPH's is described. Classes of monocarbonyls changed progressively during oxidation with respect to those present and the proportions thereof. Heating oxidized fat at 165° C. also produced great changes in the classes of monocarbonyls. Types of monocarbonyls found were saturated aldehydes, minor amounts of the two lowest molecular weight saturated methyl ketones, a mixed group which contained as a major constituent a DNPH derivative of unknown class, 2-en-1-als, and 2,4-dien-1-als. In general, with oxidation, the proportion of saturated carbonyls increased in unheated fat, and unsaturated carbonyls (particularly 2,4-dienals) increased when the fat was heated. Resolution of the classes by paper chromatography indicated the probable presence in the saturated aldehyde class of not only hexanal, propanal, ethanal, and methanol, but at least two C_7-C_9 saturated aldehydes, and acetone, methyl ethyl ketone, three carbonyls of unknown class, five 2-en-1-als, and three 2,4-dien-1-als; a total of at least 19 monocarbonyls. The dicarbonyl fraction appeared to be composed principally of α-dicarbonyls, but there was evidence of the presence of other types of dicarbonyls.

The methods described are adapted to the determination of the kind and proportions of carbonyl classes formed in small amounts of fat as the result of conditions to which the fat or fatty tissues have been exposed. Application of these methods may shed light on the mechanism of rancidity development from the standpoint of peroxide-carbonyl relationships, the well-known sensitivity of taste and the classes of carbonyls present. The potential value of the procedures in the identification and determination of individual carbonyls is indicated.

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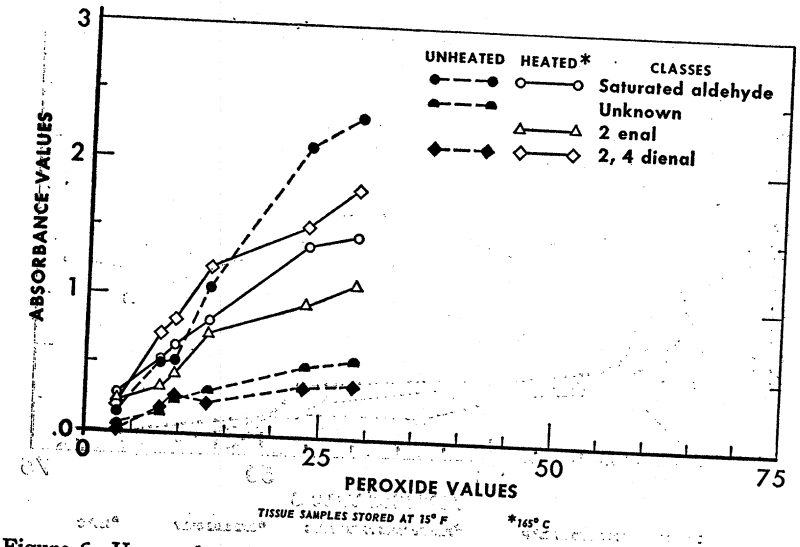

Figure 6. Uncured pork fatty tissue. Oxidation and relative amounts of monocarbonyls.

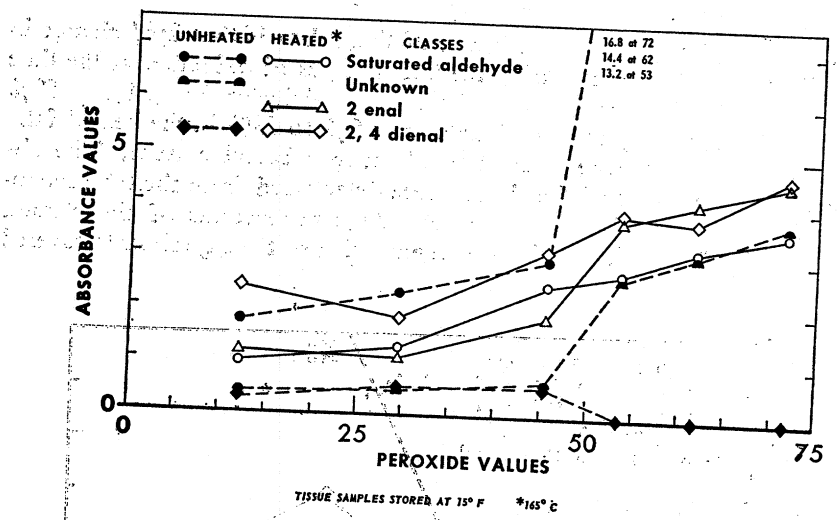

Figure 7. Cured pork fatty tissue. Oxidation and relative amounts of monocarbonyls.

by Figures 1 and 2. The absolute absorbance values are plotted against the peroxide values.

In Figure 5, the erratic behavior of the heated total and monocarbonyl discussed in connection with Figure 1 is indicated to be due primarily to the generation of 2,4-dienals in a manner unpredictable on the basis of the peroxides. The heated 2-enals also showed some variation. The rest of the class curves were fairly smooth.

In Figure 6, similar data for uncured bacon are given. The samples were from the same bacon side, and all monocarbonyl classes increased smoothly

with oxidation. These data illustrate particularly well the rapid increase and change in relative amounts of the monocarbonyl classes during the early stages of oxidation. Data shown in Figure 7 for cured bacon from the same animal (see Figure 7) are considerably different, although the fat was presumably similar in composition .Comparing Figures 6 and 7 over the same range of oxidation (peroxide 12-30), the rate of change and relative amounts of the classes are obviously different. It would appear that the pro-oxidant, salt, may influence the formation of oxidation products in some manner.

In over-all consideration of Figures 5, 6, and 7, there was a progressively increasing quantitative difference between the unheated and heated saturated classes. Although a large proportion of this class is composed of low molecular weight volatile compounds, volatilization during heating did not account for the decrease. Since the unheated saturated class increased tremendously in the late stages of oxidation, the disappearance on heating becomes very great, and probably accounts for the previously discussed reversal in quantitative relationship of the unheated and heated total monocarbonyls. Determination of the influence of fat composition and other variables on the relative amounts of the monocarbonyl classes would appear to be necessary for an understanding of the chemical nature of rancidity.

Quantitative aspects of carbonyl determination. A thorough study has been made of the reproducibility of the above determinations. This was found to usually not exceed ±3%. In the separation into classes, as shown by Gaddis and Ellis *(19)*, results for the 2,4-dienals will tend to be somewhat low and the other classes a little high. This is due to the particular sensitivity of the 2,4-dienals to light and air.

Separation of classes into individual compounds. Data shown in Figures 8 and 9 illustrate the separation by ascending paper chromatography *(13, 19)*

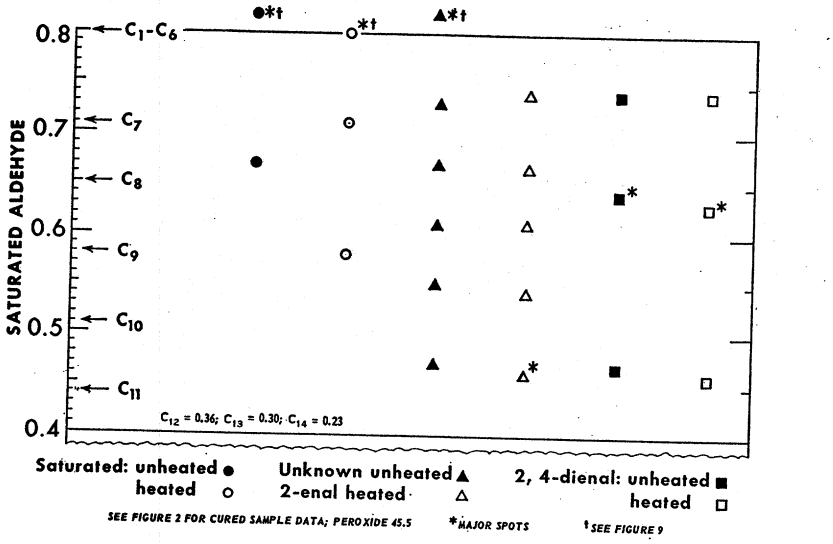

Figure 8. Vaseline-aqueous methanol system. Separation of classes into compounds, R_f values.

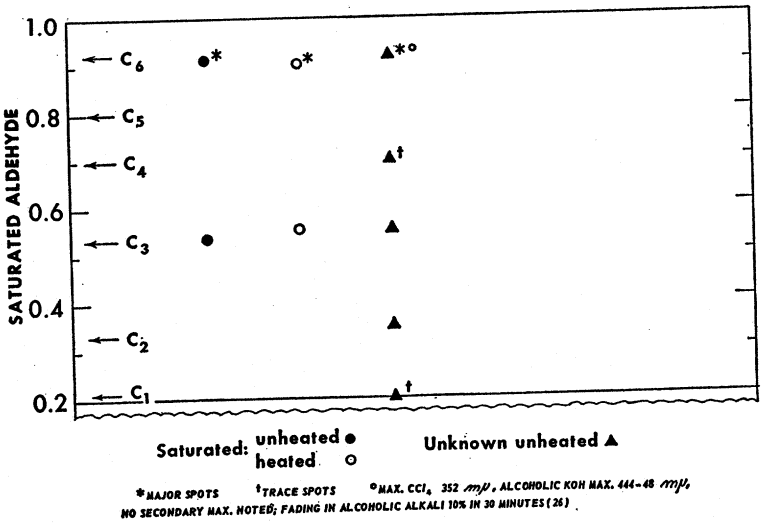

Figure 9. Propylene glycol, Skellysolve C-Methanol. R_f values of unseparated material from vaseline-methanol system.

of the different classes of a sample of unheated and heated cured pork fat into compounds. On the left-hand side, the scale indicates R_F values and the adjacent column a homologous series of authentic saturated aldehyde DNPH's. Unknown DNPH mixtures were applied first to the vaseline-aqueous methanol system (Figure 8), and after chromatography the unseparated material was transferred to the propylene glycol, Skellysolve C-methanol system (Figure 9). As indicated in Figure 8, higher molecular weight carbonyls were apparently similar in unheated and heated fats, with the exception of the saturated aldehydes. In that class, the unheated contained one compound and the heated two compounds. In the separation of lower molecular weight compounds shown in Figure 9, the heated contained none in the 2-enal class and was similar to the unheated in the saturated aldehyde class. The saturated aldehydes have been previously identified *(18)* as hexanal, propanal, ethanal, and methanal. Traces of actone and methyl ethyl ketone were also found. The two compounds with lowest R_F values in the unknown class are unquestionably methanal and ethanal *(19)*. However, the ethanal might contain acrolein and the propanal traces of acetone. These results indicate the presence of 6 saturated aldehydes, two methyl ketones, three unknown unsaturated compounds, five 2-enals, and three 2,4-dienals; a total of at least 19 monocarbonyls in oxidized pork fat.

Dicarbonyls. The solubility of the dicarbonyl fractions (Figures 1 and 2) in common organic solvents indicated they are probably mostly composed of compounds of relatively long carbon chains. The fractions had maxima in carbon tetrachloride from 350-90 mμ. Maxima of unheated dicarbonyls were generally in the range of 380-90 mμ and showed a secondary peak at 430-40 mμ. Dicarbonyls from heated fat often showed a down shift to about 350-65mμ. According to Braude and Jones (5) (see Table 1) bis hydrazones

have normal absorption except those of α diketones which have maxima as high as poly-unsaturated monocarbonyls. Apparently the downward shift in maxima indicates the presence of small amounts of unconjugated dicarbonyls. Paper chromatography *(41)* gave three main fractions with maxima at 370, 385, and 390 mμ in carbon tetrachloride. These gave purplish-blue to blue colors in alcoholic alkali with maxima at 525, 545, and 550 mμ. Several minor fractions with maxima at 346 to 358 mμ preceded the α-dicarbonyls on the paper.

SUMMARY

Methods are described for the estimation in fat of total steam volatile carbonyls, and mono- and dicarbonyl fractions thereof, as DNPH derivatives. The application of a new paper chromatographic method to the separation and determination of classes of monocarbonyl DNPH's is described. Classes of monocarbonyls changed progressively during oxidation with respect to those present and the proportions thereof. Heating oxidized fat at 165° C. also produced great changes in the classes of monocarbonyls. Types of monocarbonyls found were saturated aldehydes, minor amounts of the two lowest molecular weight saturated methyl ketones, a mixed group which contained as a major constituent a DNPH derivative of unknown class, 2-en-1-als, and 2,4-dien-1-als. In general, with oxidation, the proportion of saturated carbonyls increased in unheated fat, and unsaturated carbonyls (particularly 2,4-dienals) increased when the fat was heated. Resolution of the classes by paper chromatography indicated the probable presence in the saturated aldehyde class of not only hexanal, propanal, ethanal, and methanol, but at least two C_7-C_9 saturated aldehydes, and acetone, methyl ethyl ketone, three carbonyls of unknown class, five 2-en-1-als, and three 2,4-dien-1-als; a total of at least 19 monocarbonyls. The dicarbonyl fraction appeared to be composed principally of α-dicarbonyls, but there was evidence of the presence of other types of dicarbonyls.

The methods described are adapted to the determination of the kind and proportions of carbonyl classes formed in small amounts of fat as the result of conditions to which the fat or fatty tissues have been exposed. Application of these methods may shed light on the mechanism of rancidity development from the standpoint of peroxide-carbonyl relationships, the well-known sensitivity of taste and the classes of carbonyls present. The potential value of the procedures in the identification and determination of individual carbonyls is indicated.

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